# Effect of removing the chin gland on chin-marking behaviour in male rabbits of the New Zealand race

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> Receipt of Ms. 1. 4. 1992 Acceptance of Ms. 3. 9. 1992

#### Abstract

In male domestic rabbits the effect of removing the chin gland on the performance of chin-marking behaviour was examined and compared with the known effects of castration. The marking frequency of eight intact adult bucks was recorded for a 4-week baseline period after which the chin gland was excised. Testing was resumed two days later for a further 4, 18 or 30 weeks. Four animals were also castrated either 4 or 18 weeks after gland removal. They were then tested for a further 10 weeks without treatment, for 16 days during administration of testosterone propionate (TP), and for 2 weeks following TP withdrawal. Chinning declined by 24% within two months of gland removal in gonadally intact animals, and castration reduced this behaviour by more than half again within a month. TP administration stimulated chinning while TP withdrawal resulted in a return to pretreatment values within a week. Thus, while the chin gland and/or its secretions may modulate chin marking, gonadal androgens appear to have the major stimulatory function.

### Introduction

Rabbits (Oryctolagus cuniculus) of both sexes posses a distinctive chin gland, the secretion from which they use to mark objects in their environment by rubbing their chin over them (Black-Cleworth and Verberne 1975; Hudson and Vodermayer 1992; Lyne et al. 1964; Mykytowycz 1962). In males, both the frequency of chinning and the size and secretory activity of the gland correlate positively with social rank and reproductive activity (Bell 1985; Black-Cleworth and Verberne 1975; Mykytowycz 1962, 1965, 1972, 1975; MYKYTOWYCZ and DUDZINSKI 1966; MYKYTOWYCZ et al. 1976), and chinning is therefore thought to be involved in territorial defence, in the establishment and maintenance of the social hierarchy and in the modulation of reproductive activity.

Testicular androgens appear to play a major role in the regulation of this behaviour. Thus, whereas castration reduces the frequency of chinning (GONZÁLEZ-MARISCAL et al. 1992b; MYKYTOWYCZ 1962, 1965), the size of the gland and its histological appearance (MYKYTOWYCZ 1965; STRAUSS and EBLING 1970; WALES and EBLING 1971), administration of testosterone propionate reverses these effects (González-Mariscal et al. 1992b; MYKYTOWYCZ 1962; STRAUSS and EBLING 1970; WALES and EBLING 1971). However, as it is not known to what extent the gland itself contributes to the performance of chinning, it was the purpose of the present study to investigate the effect of removing the chin gland on

the marking behaviour of adult male rabbits.

### Material and methods

#### Animals

Eight adult New Zealand white bucks from the Tlaxcala colony, aged from 1–3 years and weighing from 3.8–4.2 kg were used. The animals were separately caged under natural light and temperature conditions (11–13 h light/day, 19  $\pm$  3 °C), with continuous access to rabbit pellets (Conejina, Purina) and water.

### Drugs

Testosterone propionate was obtained from Sigma (St. Louis, Missouri, USA).

## Behavioural testing

Chin-marking activity was assessed as described previously (HUDSON et al. 1990) by placing bucks individually for 15 min a day in a wire-mesh arena 1 m in diameter and 43 cm high, containing three, 15-cm high terracotta bricks arranged in a triangle, approximately 0.5 m apart. The number of times an animal rubbed its chin on the bricks or the arena walls during the test period was recorded. As we had earlier found that the use of either fresh bricks or those previously marked by familiar test animals did not modify chinning frequency of either male or female subjects (GONZÁLEZ-MARISCAL et al. 1992b; HUDSON and VODERMAYER 1992), the same bricks were used throughout the study. Furthermore, as animals were operated successively over several months and tested in each session in random order, subjects were presented for most of the test period with bricks marked by both intact and operated animals. By staggering the operative procedures, animals in different states could be tested in parallel, and effects due to a lack of chin marks on the bricks per se rather than to the experimental procedures themselves, could thus be minimized. Observations were conducted inside the rabbit colony between 17.00–19.00 h from September 1988 to January 1990.

### Experimental procedures

To allow animals to adapt to the experimental conditions they were introduced to the arena during the first week for 15 mins a day without recording chinning. After this period, chinning was recorded for a minimum of 3 and in most cases for 5 days a week under the following conditions: a. during a control period of four weeks, after which the chin gland was excised; b. starting 48 hours after surgery, gonadally intact animals were tested for a further 4 (N = 2), 18 (N = 2), or 30 weeks (N = 4) (cf. Fig. 1); c. four animals were castrated either 4 (N = 2) or 18 weeks (N = 2) after removal of the gland and tested for a further 10 weeks; d. following this period, castrated subjects were tested during 16 days of treatment with testosterone propionate (TP; 1 mg/day, s.c. in 1 ml sesame oil), and for 2 weeks after discontinuing TP treatment (cf. Fig. 2). A group of intact control animals was not included since we had earlier found that daily testing of both male and female rabbits over periods of two months or more does not lead to a reduction in chinning (González-Mariscal et al. 1992a, b; Hudson 1992).

### Surgery

The chin gland has been morphologically well described and is reported to consist of several discrete glandular masses including a large superficial medial group and a smaller deep lateral group situated on either side of the lower jaw (Lyne et al. 1964; Myrytowycz 1965). To remove these glands, the animals were anesthetized using 0.7 g/kg urethane administered as a 20% solution i.p. After depiliating the chin region, a 3 cm midline section was made and the skin retracted first to one side and then to the other so as to bilaterally remove the two groups of glands. In this study these were found to comprise two superficially located glandular masses encapsulated in connective tissue and readily removed, and a deeper lying, less accessible posterior mass. Surgery could be accomplished with little or no bleeding, and following excision of all visible glandular and associated connective tissue, the wound was sutured closed and an antiseptic spray (Pisan) applied locally. All wounds healed within a few days with no sign of infection.

Bilateral castration was also performed under urethane anesthesia and the wounds sutured closed and treated locally with antiseptic spray. The animals recovered rapidly with no apparent complica-

tions.

### Statistical analysis

To analyze the effect of removing the chin gland on marking activity, mean monthly values were calculated for each of the 6 bucks remaining gonadally intact to post-operative week 18, and their monthly post-operative scores compared to the pre-operative baseline values using the nonparametric, two-tailed Wilcoxon test.

#### Results

During baseline testing the animals appeared to adjust rapidly to the arena and by the second week started vigorously marking as soon as they were placed in it. Although there was considerable individual variability in the frequency of chinning, with average scores ranging between 60 and 140 marks per session, group values remained relatively stable at around 105 marks per session (Fig. 1).

#### Gland removal

Removal of the chin gland had no immediate visible effect on the marking behaviour of any of the animals. As shown in Figure 1, chinning scores remained high throughout most of the first post-operative month, with no significant differences when compared to baseline (N = 6, T = 4, p > 0.05). In fact, even on the first day of testing, that is 48 h after surgery, the vigor and frequency of chinning appeared unaltered. However, during the second month following gland removal, a significant decline in chinning from the baseline average of about 105 marks/15 min to an average of 80 marks/15 min (N = 6, T = 0, p < 0.05) was recorded. Scores then stabilized with no significant difference found between the means of the second and third months (N = 6, T = 7, p > 0.05). Furthermore, the mean chinning frequency of the four animals which remained uncastrated throughout the entire experiment varied little from week 18 (66  $\pm$  5 marks/15 min) to week 30 (79  $\pm$  6 marks/15 min) following gland removal.

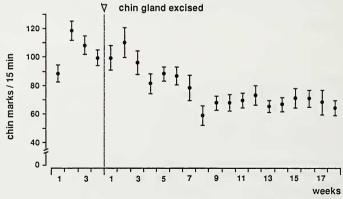


Fig. 1. Effect of removing the chin gland on the marking frequency of gonadally intact adult male rabbits. Chinning frequency was determined by placing animals in a wire-mesh arena containing three bricks, and counting the number of times animals rubbed their chin on these. Sessions lasting 15 mins were conducted three to five times a week for a four-week baseline period (N = 8), after which the chin glands were removed. Eight animals were tested to post-operative week 5, and six thereafter. Means and standard errors of the mean are given

#### Castration

Castration resulted in a clear decline in marking activity within a month (Fig. 2). This was true regardless of the time elapsing between chin-gland removal and castration. During this period, marking activity declined by an average of 50 %, after which it appeared to stabilize.

#### Testosterone administration

Daily administration of TP increased the average level of marking activity from 14 marks/15 min on day 1 of treatment to 52 marks/15 min by day 14 (Fig. 2). Discontinuing the treatment resulted in a return to pre-TP baseline values within a week, with scores declining from an average of 52 marks/15 min on the last day of TP treatment to 13 marks/15 min seven days later.

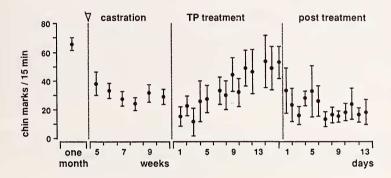


Fig. 2. Effect of castration and of androgen replacement and withdrawal on the chinning frequency of adult male rabbits following chin-gland removal. The chinning frequency of four bucks was determined in three to five, 15 minute test sessions per week under the following conditions: a. without chin glands for one month before castration; b. for ten weeks after castration; c. for 16 days with daily administration of testosterone propionate (TP; 1 mg/day); d. for 13 days after TP withdrawal. Means and standard errors of the mean are given

#### Discussion

The present results demonstrate that removal of the chin gland in gonadally intact male rabbits results in a significant decrease in chin-marking behaviour. However, the effect was slow, two months being required for a significant reduction in chinning frequency to occur. The fact that normal levels of marking were observed two days after surgery and throughout the following month makes it unlikely that the reduction was simply an artefact of the surgical procedure. As the chinning activity of intact male and female rabbits in the laboratory arena remains stable over months (GONZÁLEZ-MARISCAL et al. 1990, 1992a, b; Hudson 1992; Hudson and Vodermayer 1992), it is also unlikely that the decrease in chinning was simply due to habituation or to a decline in general motivation. Furthermore, as animals were operated and tested throughout the year, seasonal effects can also be excluded. Thus, these data would suggest that the chin gland itself or its secretions are involved in the regulation of chinning in buck rabbits. This contrasts somewhat with the study of Blum and Thiessen (1970) who found that ventral gland excision did not modify the frequency of scent-marking in male Mongolian gerbils tested five weeks after surgery. However, while species differences are quite possible, the results of the present study suggest it may be necessary to wait as long as two or three months for the effects of gland excision to become apparent.

The mechanisms mediating the decline in marking activity following chin-gland removal are unknown. However, since removal of the gland eliminates the secretions normally deposited during chinning, the decline may have been due to the failure of animals to gain confirmation of the effectiveness of the marking act. Should such olfactory feedback indeed be important in regulating chinning, then covering the gland to prevent deposition of secretion could be expected to result in a similar decline. Nevertheless, gonadal androgens appear to be the main activators of this behaviour as chin-gland excision in gonadally intact animals resulted in only a moderate decrease in marking after a long latency.

Irrespective of the mechanisms mediating the decrease in chinning following gland removal, it is clear that this effect was not as rapid or marked as following castration. Thus, while castration has been found to reduce marking by nearly 60 % within 3-4 weeks (GONZÁLEZ-MARISCAL et al. 1992b), chin-gland removal decreased chinning by only 24 % two months postoperatively.

In summary, chinning in rabbits seems to be regulated primarily by gonadal steroids. These hormones are able to stimulate chinning independently of the gland since TP stimulates marking in animals with or without chin glands, and castration reduces chinning to a similar extent in both groups. Nevertheless, the finding that chin-gland removal may also reduce the frequency of chinning suggests the regulation of this behaviour to be complex, probably involving a combination of hormonal, olfactory and somatosensory mechanisms.

### Acknowledgements

This work was supported by funds provided by the Secretaria de Education Publica de México (DGICSA: C90-01-0439; to P.P.) and the Deutsche Forschungsgemeinschaft (Hu 426/1; Po 121/13).

# Zusammenfassung

Der Einfluß der Entfernung der Kinndrüse auf das Kinn-Markierverhalten von männlichen Kaninchen der Neuseeland-Rasse

Bei männlichen Hauskaninchen wurde der Einfluß der Entfernung der Kinndrüse auf die Häufigkeit von Kinnmarkierverhalten untersucht und mit dem der Kastration verglichen. Das Markierverhalten von 8 Rammlern wurde vor Entfernung der Kinndrüse für die Dauer von 4 Wochen aufgezeichnet, und dann, 2 Tage nach dem Eingriff, für weitere 4, 18 oder 30 Wochen getestet. Die ersten 4 Tiere wurden danach zusätzlich kastriert und für weitere 10 Wochen auf ihre Kinnmarkieraktivität untersucht. Schließlich erhielten diese Tiere für 16 Tage 1 mg Testosteron-Proprionat (TP) pro Tag, und wurden dann weitere zwei Wochen beobachtet. Innerhalb von 2 Monaten nach Entfernung der Drüse sank die Markierhäufigkeit bei nicht-kastrierten Tieren um 24 %. Kastration führte zu einer zusätzlichen Abnahme um mehr als 50 % innerhalb eines Monats und Behandlung mit TP zu einer vorübergehenden Stimulation des Kinnmarkierverhaltens. Dies bedeutet, daß die Kinndrüse und/oder die Kinndrüsensekrete zwar modulierend wirken können, die Androgene das Markierverhalten aber am stärksten beeinflussen.

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